

## New England Biolabs Certificate of Analysis

**Product Name:** OneTaq® Hot Start DNA Polymerase  
**Catalog Number:** M0481X  
**Concentration:** 5,000 U/ml  
**Unit Definition:** One unit is defined as the amount of enzyme that will incorporate 15 nmol of dNTP into acid insoluble material in 30 minutes at 75°C.  
**Packaging Lot Number:** 10256527  
**Expiration Date:** 08/2026  
**Storage Temperature:** -20°C  
**Storage Conditions:** 10 mM Tris-HCl , 100 mM KCl , 1 mM DTT , 0.1 mM EDTA , 0.5 % Tween® 20 , 0.5 % IGEPAL® CA-630 , 50 % Glycerol, (pH 7.4 @ 25°C)  
**Specification Version:** PS-M0481S/L/X v2.0

OneTaq® Hot Start DNA Polymerase Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0481L	OneTaq® Hot Start DNA Polymerase	10256526	Pass

Assay Name/Specification	Lot # 10256527
<b>Inhibition of Primer Extension (Hot Start, Radioactivity Incorporation)</b> A 50 µl primer extension assay in ThermoPol® Reaction Buffer in the presence of 200 µM dNTPs including [ <sup>3</sup> H]-dTTP, containing 15 nM primed single-stranded M13mp18 with 2.5 units of OneTaq® Hot Start DNA Polymerase incubated for 16 hours at 25°C yields >95% inhibition when compared to a non-hot start control reaction.	Pass
<b>Non-Specific DNase Activity (16 Hour)</b> A 50 µl reaction in NEBuffer 2 containing 1 µg of T3 or T7 DNA in addition to a reaction containing Lambda-HindIII DNA and a minimum of 5 units of OneTaq® Hot Start DNA Polymerase incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	Pass
<b>PCR Amplification (5.0 kb Lambda DNA)</b> A 25 µl reaction in OneTaq® Standard Reaction Buffer in the presence of 200 µM dNTPs and 0.2 µM primers containing 5 ng Lambda DNA with 0.625 units of OneTaq® Hot Start DNA Polymerase for 25 cycles of PCR amplification results in the expected 5.0 kb product.	Pass
<b>PCR Amplification (Buffer Dependent, &gt;65% GC-rich)</b> A 25 µl reaction in OneTaq® GC Buffer in the presence of 200 µM dNTPs and 0.2 µM	Pass

Assay Name/Specification	Lot # 10256527
<p>primers containing 10 ng Human Genomic DNA with 0.625 units of OneTaq® Hot Start DNA Polymerase for 30 cycles of PCR amplification results in the buffer-dependent production of the expected 737 bp product.</p>	
<p><b>PCR Amplification (Enhancer Dependent, &gt;70% GC-rich)</b> A 25 µl reaction in OneTaq® GC Reaction Buffer and 20% OneTaq® High GC Enhancer in the presence of 200 µM dNTPs and 0.2 µM primers containing 10 ng Human Genomic DNA with 0.625 units of OneTaq® Hot Start DNA Polymerase for 30 cycles of PCR amplification results in the enhancer-dependent production of the expected 627 bp product.</p>	<b>Pass</b>
<p><b>PCR Amplification (Hot Start 2 kb Lambda DNA)</b> A 25 µl reaction in OneTaq® Standard Reaction Buffer in the presence of 200 µM dNTPs and 0.2 µM primers containing 10 pg Lambda DNA and 50 ng Human Genomic DNA with 0.625 units of OneTaq® Hot Start DNA Polymerase for 30 cycles of PCR amplification results in an increase in yield of the 2 kb Lambda product and a decrease in non-specific genomic bands when compared to a non-hot start control reaction.</p>	<b>Pass</b>
<p><b>RNase Activity (Extended Digestion)</b> A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 µl of OneTaq® Hot Start DNA Polymerase is incubated at 37°C. After incubation for 16 hours, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</p>	<b>Pass</b>

This product has been tested and shown to be in compliance with all specifications.

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